Journal of Medical Virology 54:54–59 (1998)

Quantification of HIV-1 Proviral DNA by a Standardized Colorimetric PCR-Based Assay

Jacques Izopet,[1]* Catherine Tamalet,[2] Christophe Pasquier,[1] Karine Sandres,[1] Bruno Marchou,[3] Patrice Massip,[3] and Jacqueline Puel[1]

[1]*Laboratoire de Virologie, Hôpital Purpan, Toulouse, France*
[2]*Laboratoire de Virologie, Hôpital La Timone, Marseille, France*
[3]*Service des Maladies Infectieuses, Hôpital Purpan, Toulouse, France*

A simple method was developed for measuring human immunodeficiency virus type 1 (HIV-1) proviral DNA in mononuclear cells based on the commercially available Amplicor(TM) HIV-1 polymerase chain reaction (PCR) assay and the limiting dilution method. The lowest limit of detection was four proviral genomes per 10^6 cells. The accuracy was demonstrated by using serial dilutions of LAV-8E5 cells, and the interassay variability was 0.2 log. The technique was used to measure HIV-1 proviral DNA in the peripheral blood mononuclear cells (PBMC) of 18 antiretroviral drug-naive HIV-1-positive individuals before and 4 weeks after initiating double nucleoside therapy. The DNA proviral titers at baseline (median = 3.45, range = 2.11–4.7 log copies/10^6 cells) were 2.08 log greater than the infectivity titers, but there was a correlation between these two parameters (r = 0.63, *P* = 0.009). The mean decrease in the proviral DNA titer after 4 weeks of therapy was 0.31 log, whereas the decrease in the infectivity titer was 0.81 log and the decrease in the plasma RNA concentration was 1.29 log. The technique was also used to measure HIV-1 proviral DNA in the PBMC of 11 patients who had undetectable plasma HIV-1 RNA after being placed on combination antiretroviral therapy. Although proviral DNA remained detectable in all patients after 36 weeks of treatment, a gradual decline with an estimated half-life of 21–58 weeks was observed. The reliability of this simple and convenient colorimetric PCR-based technique indicates its suitability for assessing the effect of current antiretroviral regimens on the latent reservoirs of provirus. ***J. Med. Virol. 54:54–59, 1998.***

KEY WORDS: HIV-1 proviral DNA; quantification; polymerase chain reaction; latent HIV-1 infection

INTRODUCTION

Recently, methods allowing measurement of human immunodeficiency virus type 1 (HIV-1) in infectious and molecular forms have been developed for studying the natural history of the infection and for assessing the efficacy of antiretroviral treatment. Cell-free HIV-1 RNA in plasma reflects directly the level of HIV-1 expression in different cells and tissues during infection. This parameter is now reliably measured by standardized commercially available assays in clinical trials and in clinical practice [Dewar et al., 1994; Mulder et al., 1994; van Gemen et al., 1994]. Cell-associated HIV-1 proviral DNA reflects the number of infected cells in the peripheral blood or in other compartments such as the lymphoid tissue. Accurate, precise, and easy measurement of HIV-1 proviral DNA could provide crucial information on the pathogenesis of HIV-1 infection and on the antiviral activity of more potent new therapeutic regimens. Investigating changes in cell-associated proviral DNA could have particular relevance in patients in whom aggressive treatment has reduced plasma HIV-1 RNA to undetectable levels.

Numerous techniques based on the polymerase chain reaction (PCR) for quantifying proviral load in HIV-infected individuals have been described. All these assays, including externally controlled PCR [Aoki et al., 1990; Bieniasz et al., 1993; Connor et al., 1993; Furtado et al., 1993; Montoya et al., 1993; Sei et al., 1994; Conway et al., 1995; Yerly et al., 1995] and internally controlled PCR with or without competition [Menzo et al., 1992; Mallet et al., 1995; Gupta et al., 1995] require specific optimization and are prone to intralaboratory or interlaboratory variations due to the lack of standardized reagents. Most of these assays also have a restricted dynamic range (<2 log), which prevents the use of a single optimized protocol for the accurate quantification of proviral DNA in different cellular

*Correspondence to: Dr. Jacques Izopet, Laboratoire de Virologie, Hôpital Purpan, CHU Toulouse, 31059 Toulouse Cédex, France. E-mail: izopet@cict.fr

Accepted 18 August 1997

compartments from the same or different individuals. The PCR products must often be quantified by radioisotope detection.

A colorimetric PCR-based assay in kit form, the Amplicor(TM) HIV-1 qualitative test (Roche Diagnostic Systems, Neuilly, France), has been used recently to detect HIV-1 proviral *gag* sequences in peripheral blood mononuclear cells (PBMC) with good analytical results [Whetsell et al., 1992; Khadir et al., 1995; Kovacs et al., 1995; Pane et al., 1995; Sethoe et al., 1995]. We have used the Amplicor(TM) kit to develop a simple semiquantitative technique for measuring HIV-1 proviral DNA in mononuclear cells, which is based on the limiting dilution method. Thus, the present study was carried out to assess the accuracy and analytical reproducibility of this technique, to determine the relationship between proviral DNA, cell-associated infectious HIV-1, and plasma HIV-1 RNA before and after nucleoside therapy, and to assess the kinetics of proviral DNA in patients on antiretroviral therapy who have undetectable plasma HIV-1 RNA.

MATERIALS AND METHODS

Serial Dilutions of LAV-8E5 Cells

The LAV-8E5 cell line containing one copy of HIV-1 provirus per cell was diluted serially in uninfected PBMC to obtain 10^4 to 10^1 copies of HIV-1 proviral DNA per 10^6 cells.

Clinical Specimens

Peripheral blood specimens were obtained with consent from 29 patients. Group I consisted of 18 consecutive HIV-1 seropositive individuals (13 men, 5 women; mean age = 39 years) who were given a nucleoside therapy for the first time: 9 patients were given zidovudine plus didanosine and 9 patients were given zidovudine plus zalcitabine. Each subject was classified using the Centers for Disease Control (CDC) system [Centers for Disease Control and Prevention, 1993] into classes A (n = 10) and C (n = 8). The mean baseline CD4 cell count was 250/mm^3 (range = 60–381). Blood samples were collected before treatment and 4 weeks after the initiation of treatment. These specimens were assayed for cell-associated HIV-1 proviral DNA, cell-associated infectious virus, and plasma HIV-1 RNA. Group II consisted of 11 selected HIV-1 seropositive individuals (8 men, 3 women; mean age = 35 years) who had undetectable plasma HIV-1 RNA at 12, 24, and 36 weeks after the initiation of combination antiretroviral therapy. They were classified as CDC classes A (n = 6), B (n = 2), and C (n = 3), and the mean baseline CD4 cell count was 242/mm^3 (range = 8–450). Cell-associated HIV-1 proviral DNA was measured retrospectively in blood specimens collected before treatment and after 12 and 36 weeks of treatment.

PBMC were isolated from blood specimens collected in heparinized tubes by Ficoll-Hypaque density. Mononuclear cells were counted and divided into several aliquots of 1×10^6 cells. The first (fresh) aliquot was used for quantitative culture. The others were stored as dry pellets at −70°C and used to measure proviral DNA. Blood specimens collected in EDTA tubes were used to measure the HIV-1 RNA in plasma.

HIV-1 Proviral DNA

HIV-1 proviral DNA was measured by endpoint dilution PCR using the Amplicor(TM) kit.

Specimen processing. An aliquot (1×10^6) of cryopreserved PBMC was thawed and suspended in 400 μl of lysis solution containing proteinase K. Fivefold serial dilutions from 5^0 to 5^{-5} were prepared from a 1.0×10^6 cells lysate using heated (100°C for 30 min) lysis solution to obtain 125,000, 25,000, 5,000, 1,000, 200, and 40 cells, each in a volume of 50 μl. PBMC from uninfected individuals were used as negative controls and the equivalent of five copies of the LAV-8E5 cell line were used as positive controls.

Amplification step. The test was carried out according to the instructions provided in the package insert of the Amplicor(TM) HIV-1 kit. Briefly, duplicate 50-μl aliquots of each cell dilution were each added to a 50-μl master mixture in a 96-well microplate. The master mixture contained N-glycosylase enzyme, Tris-HCl, dATP, dGTP, dCTP, and dUTP, biotinylated primers SK462/SK431 designed in the *gag* region [Whethsell et al., 1992], Ampli Taq DNA polymerase, and salts. Amplification was performed in a Perkin Elmer Gene Amp System 9600 thermal cycler programmed for one initial cycle at 50°C for 2 min, followed by 5 cycles at 95°C for 10 sec, 55°C for 10 sec, and 72°C for 10 sec; 30 cycles at 90°C for 10 sec, 60°C for 10 sec, and 72°C for 10 sec; and a final hold at 72°C.

Detection of PCR-amplified HIV-1 DNA. Amplified products were added to 100 μl of denaturation solution containing sodium hydroxide at room temperature for 10 min. Twenty-five microliters of denatured products of amplification were added to 100 μl of hybridization buffer into wells of a microtiter plate coated with SK102, an oligonucleotide probe internal to the HIV-1 *gag* fragment amplified with the biotinylated primers SK462/SK431. Plates were incubated at 37°C for 1 hr. Plates were then washed five times, and 100 μl of avidin-horseradish peroxidase conjugate were added to each well and incubated at 37°C for 15 min. After five washes, 100 μl of tetramethylbenzidine and hydrogen peroxide were added in each well. The microtiter plate was protected from light and left at room temperature for 10 min. Degradation of substrate was stopped with the addition of 100 μl of 1 N H_2SO_4. The absorbance of each well at A_{450} was recorded in a Beckman spectrophotometer. The detection cutoff for positivity was 0.35.

Calculation of titers. The proviral DNA titers are expressed as the number of proviral genomes per 10^6 cells. The copy number is determined by limiting-dilution analysis and dilution assay statistics based on the method of maximum likelihood. The critical assumption that underlies the use of Poisson analysis is that signal-generating units are distributed at random within each sample. The copy number is determined by

analyzing the likelihood matrix. Each of the 12 wells (6 fivefold dilutions in duplicate) can conceivably be either positive or negative, but a large majority of the outcomes are in fact very unlikely to occur. For instance, 221,000 indicates that both wells are positive at the first level, both wells are positive at the second level, one of two wells is positive at the third level, and all wells are negative at the other levels; the corresponding estimate is 130 proviral genomes per 10^6 cells. The calculations were carried out by a computer program developed by INSERM (Service Commun 10, Paris, France). The lowest measurable proviral DNA titer was 4 proviral genomes per 10^6 cells.

Infectious HIV-1

The coculture technique recommended by the French Agency for AIDS Research [Rouzioux et al., 1992] was used. Briefly, decreasing numbers of fresh PBMC (10^6, 2×10^5, 4×10^4, 8×10^3, 1.6×10^3, 3.2×10^2) were cultured in duplicate with 2×10^6 phytohemagglutinin-stimulated donor PBMC in 24-well plates. Each well contained 1.5 ml RPMI 1640 medium, 10% fetal calf serum, and 20 IU/ml native interleukin-2 (Biotest, Frankfurt, Germany). Half the medium was renewed twice a week, and 3×10^5 preactivated normal PBMC were added once a week. The cultures were maintained for 3 weeks, and virus production was determined by assaying the p24 antigen in the medium on day 21. Results are expressed as the number of infectious units (NIU) per 10^6 cells.

HIV-1 RNA

The HIV-1 RNA in plasma was measured with the Amplicor(TM) HIV-1 Monitor(TM) reverse transcriptase-PCR (RT-PCR) assay (Roche Diagnostic Systems) according to the manufacturer's instructions. The cutoff value of this assay is 200 copies/ml.

CD4 Lymphocyte Counts

Peripheral blood CD4 lymphocytes were counted by flow cytometry (Epics Profile; Coulter, Hialeah, FL) using commercially available monoclonal antibodies (Beckton Dickinson, Mountain View, CA).

Statistical Analysis

Proviral DNA titers, infectivity titers, and HIV-1 RNA concentrations were transformed to log (base 10) values before analysis. Specimens in which HIV-1 RNA was undetectable were assigned values equal to the detection limit of the Monitor RT-PCR assay (i.e., 2.3 log). Specimens in which infectious virus was undetectable were assigned a value of zero because the lowest measurable infectivity titer was <1 infectious unit per 10^6 cells. Interassay variability is expressed as the standard deviation of the log-transformed copy number of replicate samples. Correlations among variables were computed with Spearman's rank correlation. The Wilcoxon test was used to compare paired values. Repeated measures were analyzed by the Friedman test to check for the presence or the absence of a significant variation during the follow-up period. A P value of less than 0.05 was considered to be significant.

TABLE I. Quantification of HIV-1 Proviral DNA in 10-Fold Serial Dilutions of LAV-8E5 Cells in Uninfected Peripheral Blood Mononuclear Cells

	HIV-1 proviral DNA (copies/10^6 cells)	
LAV-8E5 cells/10^6 cells	Run 1	Run 2
10,000	7,943	7,943
1,000	654	324
100	65	65
10	26	4
0	0	0

RESULTS

Analytical Performance of HIV-1 Proviral DNA Quantification

The HIV-1 proviral DNA was measured on 10-fold serial dilutions of LAV-8E5 cells to obtain 10^4 to 10^1 copies of HIV-1 DNA per 10^6 cells. The titers obtained for each dilution in two different runs were similar, ranging from 7,943 to 4 copies per 10^6 cells. The dilution curves were linear over the range of concentrations tested (Table I). The interassay variability of the proviral DNA quantification was assessed by testing five aliquots of 1×10^6 cells from two clinical specimens in five separate experiments (Table II). The standard deviations of log copies were 0.14 log and 0.21 log. Similar values were obtained by duplicate testing of additional clinical specimens (data not shown).

Relationships Between Proviral DNA, Cell-Associated Infectious HIV-1, and Plasma HIV-1 RNA

Blood specimens from 18 drug-naive patients (group I) were assayed for proviral DNA, cell-associated infectious HIV-1, and plasma HIV-1 RNA before and 4 weeks after the initiation of double nucleoside therapy (zidovudine plus didanosine or zalcitabine) (Table III). The baseline proviral DNA was measured in the PBMC of all subjects, cell-associated infectious HIV-1 was detected in 17/18 subjects, and HIV-1 RNA was detected in all plasma samples. The median proviral DNA titer was 3.45 log copies/10^6 cells (range = 2.11–4.70), the median infectious titer was 1.43 log NIU/10^6 cells (range = 0–2.45), and the median plasma HIV-1 RNA concentration was 4.18 log copies/ml (range = 3.14–5.28). There was a correlation between the proviral DNA titer and the cell-associated infectious titer ($r = 0.63$, $P = 0.009$) (Fig. 1) or the plasma HIV-1 RNA concentration ($r = 0.63$, $P = 0.009$). The difference between the proviral DNA titer and the infectivity titer was 2.08 ± 0.54 log. There was no correlation between this difference and either the proviral DNA titer or the infectivity titer.

The median proviral DNA titer in PBMC was 3.22 after 4 weeks of treatment, which was a mean of 0.31 log lower than baseline values ($P = 0.015$). Cell-

TABLE II. Determination of Interassay Variability of HIV-1 Proviral DNA Quantification on Two Clinical Specimens

Specimen	HIV-1 proviral DNA (copies/10^6 cells) Run 1	Run 2	Run 3	Run 4	Run 5	Mean (log copies)	SD (log copies)
1	65	65	130	36	65	1.87	0.14
2	3,364	1,647	3,364	1,647	1,143	3.30	0.21

TABLE III. Proviral DNA, Cell-Associated Infectious Virus, and Plasma HIV-1 RNA Before and After 4 Weeks of Double Nucleoside Therapy in 18 Drug-Naive HIV-1-Positive Individuals

Patient	Clinical Characteristics: CDC stage	Conditions	CD4+ cell counts at baseline (/mm^3)	HIV-1 proviral DNA (log copies/10^6 cells): Baseline	Week 4	Infectious HIV-1 (log NIU/10^6 cells): Baseline	Week 4	HIV-1 RNA (log copies/ml): Baseline	Week 4
1	A	Asymptomatic	350	3.22	2.51	0.56	—	3.38	<2.30
2	A	Asymptomatic	296	4.04	3.22	1.35	—	4.08	<2.30
10	A	Asymptomatic	205	4.70	4.70	1.37	1.92	5.15	4.94
11	A	Asymptomatic	217	4.30	3.53	2.25	0.52	4.98	2.60
12	A	Asymptomatic	212	3.91	3.22	1.53	0.52	3.82	2.45
3	A	Lymphadenopathy	260	3.36	2.51	1.02	—	3.33	2.38
4	A	Lymphadenopathy	327	3.22	2.93	1.18	—	4.04	2.76
13	A	Lymphadenopathy	381	2.67	2.11	0.42	—	3.32	3.06
14	A	Lymphadenopathy	234	2.11	2.11	—	—	3.85	<2.30
15	A	Lymphadenopathy	183	3.53	3.78	2.09	0.41	4.93	3.90
5	B	Herpes Zoster	348	3.37	2.51	0.66	—	4.47	<2.30
6	C	Kaposi's sarcoma	300	4.04	3.90	2.12	2.08	5.05	4.47
7	B	Hairy leukoplasia	268	2.82	3.22	1.38	0.64	4.29	<2.30
8	C	Mycobacterium tuberculosis	219	3.06	2.82	1.47	0.56	3.14	<2.30
9	C	Herpes simplex, >1 month old	60	4.04	3.78	2.08	1.28	5.15	2.51
16	B	Weight loss	280	3.90	4.30	2.20	0.48	3.74	3.03
17	C	Kaposi's sarcoma	254	3.91	3.22	2.45	1.87	4.59	4.36
18	C	Cytomegalovirus, colitis	200	3.37	3.53	1.93	1.30	4.28	3.05

[a]CDC, Centers for Disease Control; NIU, number of infectious units.

associated infectious HIV-1 was detected in 11/18 (61%) cases at week 4, with a median infectivity titer of 0.5 (vs. baseline values, $P = 0.0005$). Plasma HIV-1 RNA was detected in 12/18 (67%) cases at week 4, with a median concentration of 2.55 (vs. baseline values, $P = 0.0002$). Thus, there was a mean decrease at week 4 of 0.81 log in the infectivity titer and a decrease of 1.29 log in the plasma HIV-1 RNA concentration. The difference between the proviral DNA titer and the infectivity titer after 4 weeks of nucleoside therapy was 2.58 ± 0.60 log.

Change in HIV-1 Proviral DNA in Patients Who Had Undetectable Plasma HIV-1 RNA Under Combination Therapy

HIV-1 proviral DNA was measured on sequential samples from 11 patients (group II) who began combination antiretroviral therapies and had undetectable plasma HIV-1 RNA, as assessed by the RT-PCR Monitor assay at 12, 24, and 36 weeks of treatment (Table IV). The DNA proviral titers declined significantly during the follow-up period ($P < 0.001$). A DNA proviral decrease of >0.5 log was noted in 6/11 patients after 12 weeks of treatment and in 8/11 patients after 36 weeks. A marked decrease of >1 log by week 36 was observed in 4 of the 5 patients on the three-drug therapy that included a protease inhibitor, whereas the 3 patients on the two-drug therapy had a reduction rate of proviral DNA <0.5 log. The rates of proviral DNA decay were analyzed by linear regression. Exponential decline in titers was demonstrated by a straight-line fit to the data on a log plot; the slope of this line permitted the half-life t½ of proviral DNA decay to be determined. The estimated t½ varied from 21 to 58 weeks.

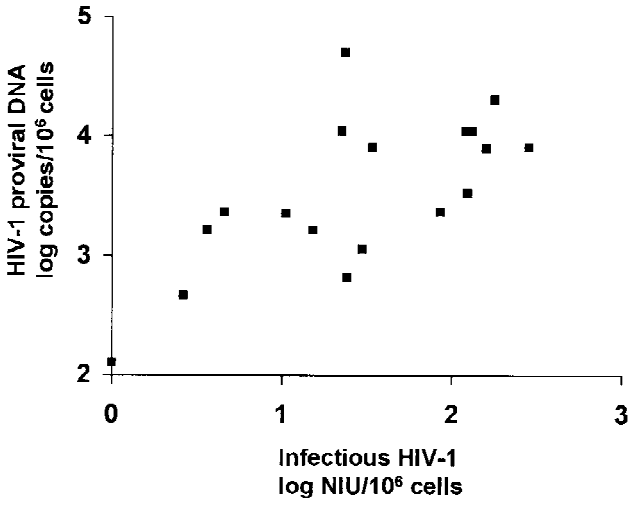

Fig. 1. Relationship between proviral DNA titers and cell-associated infectious titers of 18 antiretroviral drug-naïve HIV-1-positive individuals. Spearman test: $r = 0.63$, $P = 0.009$.

TABLE IV. Changes in HIV-1 Proviral DNA in Patients With Undetectable Plasma HIV-1 RNA After Initiating a Combination Therapy[a]

Patient	Therapy	Characteristics at baseline		CD4 (/mm^3)			Proviral DNA (log copies/10^6 cells)		
		Clinical stage	RNA (log copies/ml)	Baseline	Week 12	Week 36	Baseline	Week 12	Week 36
1	AZT + ddC + Ritonavir	C	4.37	120	253	170	4.30	3.90	3.22
2	AZT + ddC + Ritonaivir	C	4.88	281	278	279	4.30	3.53	3.06
3	AZT + 3TC + Indinavir	B	6.47	10	99	240	3.90	3.06	2.81
4	AZT + 3TC + Indinavir	C	4.98	8	9	3	3.53	3.22	2.81
5	AZT + ddC + Indinavir	A	3.77	32	49	52	3.53	2.81	2.35
6	AZT + ddl + 3TC	B	4.03	275	367	208	3.53	2.81	2.81
7	AZT + ddl + 3TC	A	4.73	430	869	725	3.06	2.51	2.51
8	AZT + ddl + 3TC	A	4.78	377	439	532	3.53	3.06	2.81
9	AZT + ddC	A	2.76	450	532	490	1.81	1.81	1.41
10	AZT + ddC	A	4.27	419	411	481	3.53	3.22	3.06
11	AZT + ddC	A	3.27	259	371	318	1.81	1.81	1.41

[a]AZT, zidovudine; ddC, zalcitabine; ddl, didanosine; 3 TC, lamivudine.

DISCUSSION

Recent advances in the development of standardized molecular biology techniques have provided valuable tools for accurate and reproducible measurement of plasma HIV-1 RNA. These assays have helped in the evaluation of new antiviral therapies and in studies on fundamental aspects of disease pathogenesis and are used now to manage infected individuals in clinical practice [Saag et al., 1996]. As combination therapies produce massive reductions in the HIV-1 RNA in plasma, attention has begun to focus on the cell-associated HIV-1 proviral DNA. However, there is as yet no standardized quantitative assay for measuring proviral DNA. We therefore developed a semiquantitative assay using the Amplicor(TM) HIV-1 qualitative test. This standardized colorimetric PCR assay has been shown to perform well in terms of sensitivity, specificity, and technical flexibility [Whetsell et al., 1992; Khadir et al., 1995; Kovacs et al., 1995; Pane et al., 1995; Sethoe et al., 1995]. The accuracy of our limiting dilution method was checked by using serial dilutions of LAV-8E5 cells, each containing one copy of HIV-1 provirus per cell. The interassay variability was approximately 0.2 log, which is similar to the analytical reproducibility of HIV-1 RNA assays [Lin et al., 1994].

The relationship between the number of circulating infected cells and the number of circulating in vitro virus-producing cells is still not accurately known. These two parameters were both measured in the present study by using the limiting dilution method with fivefold dilutions, thus allowing a direct comparison. There was a correlation between the proviral DNA titer and the infectivity titer, but the proviral DNA titer was much greater than the infectivity titer, with a mean difference of 2.08 log. This difference was quite similar in all patients and did not appear to be associated with the clinical stage. These data are consistent with the recent demonstration [Chun et al., 1997] that the most prevalent form of HIV-1 DNA in resting and activated CD4+ cells is an unintegrated form that is not replication competent. The mean difference between the proviral DNA titer and the infectivity titer was 0.5 log greater after 4 weeks of treatment than the baseline difference. Indeed, there was a smaller reduction in the proviral DNA titer after 4 weeks of treatment than in the infectivity titer or in the plasma HIV-1 RNA concentration. This difference between the loss of proviral DNA and cell-associated infectious virus in response to therapy could be due to the response in lymphoid tissues, where mononuclear cells with the highest intracellular concentrations of HIV-1 turn over rapidly and disappear quickly with treatment [Cavert et al., 1997].

The changes in proviral DNA in patients on effective antiretroviral therapy were assessed by taking and analyzing sequential blood samples from 11 patients with undetectable plasma HIV-1 RNA. Proviral DNA remained detectable in all cases after 36 weeks of treatment. However, there was a gradual decline, with an estimated half-life in good agreement with the recent findings of Perelson et al. [1997] who used a mathematical model to show that the proviral DNA decay has a mean t½ of 145 days (range = 21–433). Clearly, further follow-up is needed to determine whether the proviral DNA decay continues to indicate that viral eradication is possible.

In summary, a simple, standardized, accurate, and reproducible technique was developed for measuring HIV-1 proviral DNA in any given subject over a 3 log range without adjustment of sample size. This method should be of value in studying aspects of HIV-1 pathogenesis by using measurements of proviral DNA in blood and other compartments such as the lymphoid tissue and for assessing the effect of potent antiretroviral regimens on the latent reservoirs of provirus.

ACKNOWLEDGMENTS

We thank Eliane Coutanceau and Laurence Simet for secretarial assistance. We thank Dr. Bertrand Aulong and Dr. Chalom Sayada (Roche Diagnostic Systems, France) for providing Amplicor(TM) kits. We also thank Michèle Cazabat for technical assistance.

REFERENCES

Aoki S, Yarchoan R, Thomas RV, Pluda JM, Marczyk K, Broder S, Mitsuya H (1990): Quantitative analysis of HIV-1 proviral DNA in peripheral blood monuclear cells from patients with AIDS or ARC: Decrease of proviral DNA content following treatment with 2′, 3′-dideoxyinosine (ddI). AIDS Research and Human Retroviruses 6:1331–1339.

Bieniasz PD, Ariyoshi K, Bourelly MAD, Bloor S, Foxall RB, Harwood EC, Weber JN (1993): Variable relationship between proviral DNA load and infectious virus titre in the peripheral blood monuclear cells of HIV-1-infected individuals. AIDS 7:803–806.

Cavert W, Notermans DW, Staskus K, Wietgrefe SW, Zupancic M, Gebhard K, Henry K, Zhang ZQ, Mills R, McDade H, Goudsmit J, Danner SA, Haase AT (1997): Kinetics of response in lymphoid tissues to antiretroviral therapy of HIV-1 infection. Nature 276: 960–964.

Centers for Disease Control and Prevention (1993): Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. Morbidity and Mortality Weekly Report 41:1–19.

Chun TW, Carruth L, Finzi D, Shen X, DiGiuseppe JA, Taylor H, Hermankova M, Chadwick K, Margolick J, Quinn TC, Kuo YH, Brookmeyer R, Zeiger MA, Barditch-Crovo P, Siliciano RF (1997): Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature 387:183–188.

Connor RI, Mohri H, Cao Y, Ho DD (1993): Increased viral burden and cytopathicity correlate temporally with CD4+ T-lymphocyte decline and clinical progression in human immunodeficiency virus type 1-infected individuals. Journal of Virology 67:1772–1777.

Conway B, Ko DS, Cameron W (1995): Quantitative PCR for the measurement of circulating proviral load in HIV-infected individuals. Clinical and Diagnostic Virology 3:95–104.

Dewar RL, Highbarger HC, Sarmiento MD, Todd JA, Vasudevachari MB, Davey RT, Kovacs JA, Salzman NP, Lane HC, Urdea MS (1994): Application of branched DNA signal amplification to monitor human immunodeficiency virus type 1 burden in human plasma. Journal of Infectious Diseases 170:1172–1179.

Furtado MR, Murphy R, Wolinsky SM (1993): Quantification of human immunodeficiency virus type 1 tat mRNA as a marker for assessing the efficacy of antiretroviral therapy. Journal of Infectious Diseases 167:213–216.

Gupta P, Ding M, Cottrill M, Rinaldo C, Kingsley L, Wolinsky S, Mellors J (1995): Quantitation of human immunodeficiency virus type 1 DNA and RNA by a novel internally controlled PCR assay. Journal of Clinical Microbiology 33:1670–1673.

Khadir A, Coutlee F, Saint-Antoine P, Olivier C, Voyer H, Kessous-Elbaz A (1995): Clinical evaluation of amplicor HIV-1 test for detection of human immunodeficiency virus type 1 proviral DNA in peripheral blood mononuclear cells. Journal of Acquired Immune Deficiency Syndromes 9:257–263.

Kovacs A, Xu J, Rasheed S, Li X, Kogan T, Lee M, Liu C, Chan L (1995): Comparison of a rapid nonisotopic polymerase chain reaction assay with four commonly used methods for the early diagnosis of human immunodeficiency virus type 1 infection in neonates and children. Pediatric Infectious Diseases Journal 14:948–954.

Lin HJ, Myers LE, Yen-Lieberman B, Hollinger FB, Henrard D, Hooper CJ, Kokka R, Kwok S, Rasheed S, Vahey M, Winters MA, McQuay LJ, Nara PL, Reichelderfer P, Coombs RW, Jackson JB (1994): Multicenter evaluation of quantification methods for plasma human immunodeficiency virus type 1 RNA. Journal of Infectious Diseases 170:553–562.

Mallet F, Hebrard C, Livrozet JM, Lees O, Tron F, Touraine JL, Mandrand B (1995): Quantitation of human immunodeficiency virus type 1 DNA by two PCR procedures coupled with enzyme-linked oligosorbent assay. Journal of Clinical Microbiology 33: 3201–3208.

Menzo S, Bagnarelli P, Giacca M, Manzin A, Varaldo PE, Clementi M (1992): Absolute quantitation of viremia in human immunodeficiency virus infection by competitive reverse transcription and polymerase chain reaction. Journal of Clinical Microbiology 30: 1752–1757.

Montoya JG, Wood R, Katzenstein D, Holodny M, Merigan TC (1993): Peripheral blood mononuclear cell human immunodeficiency virus type 1 proviral DNA quantification by polymerase chain reaction: Relationship to immunodeficiency and drug effect. Journal of Clinical Microbiology 31:2692–2696.

Mulder J, McKinney N, Christopherson C, Sninsky J, Greenfield L, Kwok S (1994): Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: Application to acute retroviral infection. Journal of Clinical Microbiology 32:292–300.

Pane F, Butto S, Gobbo ML, Franco M, Butteroni C, Pastore L, Maiorano G, Foggia M, Tullio Cataldo P, Guarino A, Tamburrini E, Solinas S, Piazza M, Vecchio G, Verani P, Salvatore F (1995): Direct detection of proviral gag segment of human immunodeficiency virus in peripheral blood lymphocytes by colorimetric PCR assay as a clinical laboratory tool applied to different At-risk populations. Journal of Clinical Microbiology 33:641–647.

Perelson AS, Essunger P, Cao Y, Vesanen M, Hurley A, Saksela K, Markowitz M, Ho DD (1997): Decay characteristics of HIV-1-infected compartments during combination therapy. Nature 387: 188–191.

Rouzioux C, Puel J, Agut H, Brun-Vézinet F, Ferchal F, Tamalet C, Descamps P, Fleury H (1992): Comparative assessment of quantitative HIV viraemia assays. AIDS 6:373–377.

Saag MS, Holodniy M, Kuritzkes DR, O'Brien WA, Coombs R, Poscher ME, Jacobsen DM, Shaw GM, Richman DD, Volberding PA (1996): HIV viral load markers in clinical practice. Nature Medicine 2: 625–629.

Sei S, Kleiner DE, Kopp JB, Chandra R, Klotman PE, Yarchoan R, Pizzo PA, Mitsuya H (1994): Quantitative analysis of viral burden in tissues from adults and children with symptomatic human immunodeficiency virus type 1 infection assessed by polymerase chain reaction. Journal of Infectious Diseases 170:325–333.

Sethoe SY, Ling AE, Sng EH, Monteiro EH, Chan RKW (1995): PCR as a confirmatory test for human immunodeficiency virus type 1 infection in individuals with indeterminate western-blot (Immunoblot) profiles. Journal of Clinical Microbiology 33:3034–3036.

Van Gemen B, van Beuningen R, Nabbe A, van Strijp D, Jurriaans S, Lens P, Kievits T (1994): A one-tube quantitative HIV-1 RNA NASBA nucleic acid amplification assay using electrochemiluminescent (ECL) labelled probes. Journal of Virological Methods 49: 157–168.

Yerly S, Kaiser L, Baumberger C, Hirschel B, Perrin LH (1995): Early and prolonged decrease of viremia in HIV-1-infected patients treated with didanosine. Journal of Acquired Immune Deficiency Syndromes 8:358–364.

Whetsell AJ, Drew JB, Milman G, Hoff R, Dragon EA, Adler K, Hui J, Otto P, Gupta P, Farzadegan H, Wolinsky SM (1992): Comparison of three nonradioisotopic chain reaction-based methods for detection of human immunodeficiency virus type 1. Journal of Clinical Microbiology 30:845–853.